A new species of *Belocaulus* (Gastropoda: Veronicellidae) from southern and southeastern Brazil

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ABSTRACT

A new species is proposed based on material collected in the states of Minas Gerais, São Paulo, Santa Catarina, and Rio Grande do Sul, Brazil. The morphology, radula, and jaw are described and illustrated. The new species is compared to *Belocaulus angustipes*, the only species of the genus currently considered valid. The main differences are found in the penis. The new species has small projections, similar to tubercles, on the anterior region of the glans, which can be scattered or arranged in two, three or more longitudinal rows. The terminal extremity of the glans has digitiform margin. The penis base is short and poorly defined. The accessory gland is completely immersed in the tegument. The description of the new species extends the distribution range of *Belocaulus* for the states of Minas Gerais and São Paulo, Brazil.

 $\label{eq:Additional keywords: Veronicellidae, Belocaulus, morphology, land slug$

INTRODUCTION

Veronicellidae includes terrestrial slugs of the subclass Gymnophila with pantropical distribution, with no shell, and no developed pulmonary cavity such as that observed in other terrestrial slugs of the subclass Pulmonata. Some genera have been better studied because they include intermediate host species for the nematodes Angiostrongylus costaricensis Moreira and Céspedes, 1971, and Augiostrougylus cantonensis (Chen, 1935), parasites responsible for abdominal angiostrongiliasis and cosinophilic meningoencephalitis, respectively. Among the veronicellid species cited as intermediate hosts for A. costaricensis are Sarasinula plebeia (Fisher, 1868) in Central and South America, Phyllocaulis variegatus (Semper, 1885), Phyllocaulis soleiformis (d'Orbigny, 1835), Sarasinula linguaeformis (Semper, 1885), and Belocaulus angustipes (Heynemann, 1885) in southern Brazil, and for A. cantoneusis, Sarasinula marginata (Semper, 1885) in the state of Espírito Santo, Brazil (Graeff-Teixeira et al., 1989; Graeff-Teixeira et al., 1994; Rambo, 1997; Laitano et al., 2001; Caldeira et al., 2007). Veronicellid species have also been cited as damaging to agricultural crops (Pereira and Gonçalves, 1949; Araújo, 1952; Santos, 1959; Thomé, 1993; Milanez and Chiaradia, 1999; Chiaradia et al., 2004; Robinson and Hollingsworth, 2004).

When Hoffmann (1925) proposed *Belocaulus*, he included in the genus South American species that presented the following characters: penis shaped as an asymmetrical arrow or irregularly widened, vas deferens opening terminally or subterminally, and presence of a small accessory gland behind the duct of the bursa copulatrix and rectum, partially covered by the tegument. That author included six species in the genus without, however, designating a type species: *B. langsdorfi* (Férussac, 1822), *B. boetzkesi* (Miller, 1879), *B. pterocaulis* (Simroth, 1913), *B. festae* (Colosi, 1921), *B. pulcher* (Colosi, 1921) and *B. sloanei* (Cuvier, 1817). Baker (1925) designated *Vaginulus angustipes* as the type species of *Belocaulus* and regarded the latter as a synonym of *Angustipes* Colosi, 1922.

The synonymy proposed by Baker (1925) was not accepted by Thomé (1975), who revised the neotropical species of Veronicellidae. According to Thomé (1975), Belocaulus is valid and characterized by the presence of a small accessory gland between the rectum and the female genital pore (which, according to Thomé, is absent in Angustipes). He mentioned additional characteristics of Belocaulus: the rectum penetrates close to the female genital pore, the penial gland has uniform and sinuous tubules at the base (where they are enveloped as a whole by a membrane), and the bursa copulatrix has a kidney or oval shape, with a short duct and other connecting duct that penetrates at the base of the gland accessory.

The six species included in *Belocaulus* by Hoffmann (1925) were transferred to other Neotropical genera by Thomé (1975): *Novovaginula* Thiele, 1931, *Simrothula* Thomé, 1975, *Colosius* Thomé, 1975, and *Veronicella* Blainville, 1817. According to Thomé (1975), *Belocau*-

lus included two valid species: B. angustipes, described originally from Taquara (State of Rio Grande do Sul), and B. aberrans (Heynemann, 1885), described originally from Santa Cruz do Sul (State of Rio Grande do Sul). However, Pitoni and Thomé (1981) and more recently Thomé (1993) regarded B. aberrans as a synonym of B. angustipes, rendering Belocaulus monotypic.

Belocaulus angustipes merits special attention because of its wide distribution in southern South America (east of the Andes). It is recorded from Rio Grande do Sul and Santa Catarina, in Brazil, as well as from Uruguay, Argentina, and Paraguay (Pitoni and Thomé, 1981; Thomé, 1993; Thomé et al., 1999; Simone, 2006; Thomé et al., 2006) (Figure 1). Belocaulus angustipes is also recorded from Honduras and south of the United States where it is considered an introduced species (Thomé, 1989; Caballero et al., 1991; Thomé, 1993); the species distribution is discontinuous.

The study of a large number of specimens of *Belocaulus* from twelve localities revealed the existence of a new species, which distributed throughout southern and southeastern Brazil. Its morphology is very similar to that of *B. angustipes*, although both species can be distinguished from each other by some characters of the male reproductive system. The morphology, radula, and jaw of the new species are described and illustrated. It is also compared to *B. angustipes*, and the main differences and similarities are pointed out. New records of *Belocaulus* are provided in the states of Minas Gerais and São Paulo.

MATERIALS AND METHODS

The description of the new species proposed herein is based on the examination of 92 specimens from 35 lots collected from twelve different localities in southern Brazil. The material is deposited in the collections of the Museu de Zoologia, Universidade de São Paulo (MZUSP) (lots 87747, holotype, 87748–87750, para-



Figure 1. Map showing the distribution of *Belocaulus willibaldoi* new species and *Belocaulus angustipes*, considering literature records and lots recently collected of the latter species (from Caxias do Sul and Pinhal).

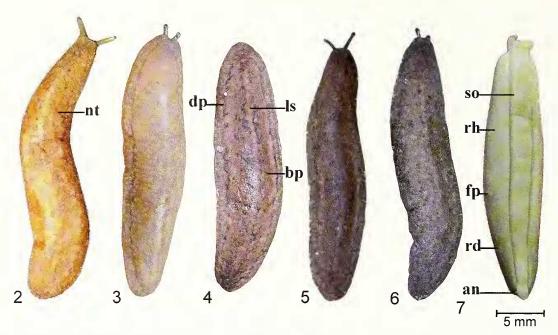
types), Museu de Ciências e Tecnologia, Universidade Católica do Rio Grande do Sul (MCP) (lots 7971, 7972), and in the Malacology collection of the Superintendência de Controle de Endemias, São Paulo, (SUCEN) (lots 8968, 8982-84, 8987-8995, 8997, 9005, 9006, 9016, 9019, 9020, 9031-9037, 9039, 9042, and 9043). Specimens of B. angustipes from Caxias do Sul, Pinhal, and Santa Maria, Brazil, and Santa Fé and Tucumán, Argentina were also examined for comparison. These are deposited at SUCEN (lots 9021, 9022, 9029, 9030 and 9038). Most of the material is preserved in 70% ethanol, although some of the material from São Paulo was fixed in Raillet-Henry. Animals were killed by submersion in filtered water, and kept in hermetically-closed recipients in the refrigerator for 48-72 hours. Prior to fixation, specimens were photographed using a Canon Digital Power Shot SD630 and observed alive. Preserved specimens were dissected under a stereomicroscope. Anatomical illustrations were made using a camera lucida. Pictures of the internal structures were obtained with a DFC 280 digital camera attached to the stereomicroscope. Digital images were merged using Automontage Pro (Synchroscopy) and Zeiss LSM Browser. Five radulae and five jaws of the new species were extracted and examined under a scanning electron microscope LEO 440 at the Museu de Zoologia, Universidade de São Paulo (MZUSP) (lots 8997, 9005, and 8983). The terminology and anatomical characters described and illustrated are in accordance to those considered diagnostic for Veronicellidae according to Thomé et al. (2006), Gomes et al. (2006) and Gomes (2007).

RESULTS

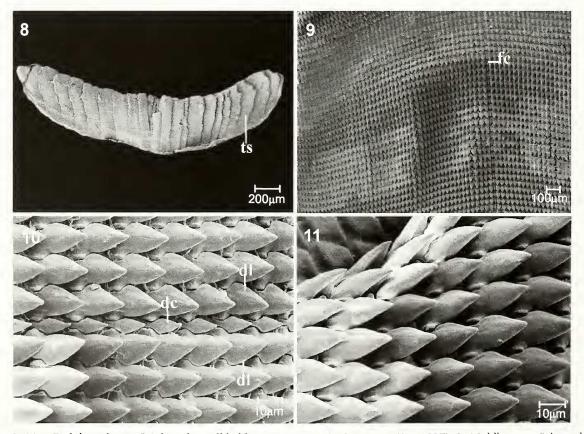
Belocaulus willibaldoi new species

Diagnosis: Belocaulus willibaldoi bears small projections similar to tubercles in the anterior region of the glans. These can be uneven or arranged in two, three, or more longitudinal rows. The glans presents a widened basal region, narrowing towards the extremity and ending in a digitiform margin. The penis base is short and poorly defined. The accessory gland is completely immersed in the tegument.

External Morphology: The length of the examined specimens ranges from 2.19 to 7.1 cm, the total width from 0.63 to 1.80 cm, the sole width from 0.16 to 0.52 cm, the left hyponotum width from 0.28 to 0.90 cm, and the right hyponotum width from 0.32 to 0.90 cm. Notum coloration varies from brown to light or grayish-brown (Figures 2–7). Mostly dark, slightly or strongly conspicuous black puncta are noticeable, usually scattered. In the majority of the specimens, there is a lighter, median, longitudinal stripe on the notum. In specimens with a darker notum, coloration pattern tends to be more uniform with slightly conspicuous puncta and lighter median, longitudinal stripe. The hyponotum and the sole are beige. The external borders of the hyponotum



Figures 2–7. External view of different forms of *Belocaulus willibaldoi* new species. **2–6.** Dorsal view showing the variation patterns of coloration. **7.** Ventral view. Abbreviations: **an:** anus position; **bp:** black puncta; **dp:** dotted line delimitating the perinotum; **fp:** female genital pore; **ls:** lighter, median, longitudinal stripe; **nt:** notum; **rd:** region of darker pigmentation; **rh:** right hyponotum; **so:** sole.



Figures 8–11. Radula and jaw of *Belocaulus willibaldoi* new species. **8.** Entire jaw (Lot 8997). **9.** Middle part of the radula (lot 8997). **10.** Central part of the radula, showing lateral and central teeth (lot 8983). **11.** Lateral teeth fit (lot 8983). Abbreviations: **de:** central teeth; **dl:** lateral teeth; **fc:** rows of central teeth; **ts:** transversal stripes.

can present a narrow stripe of darker pigmentation. There is a dotted line delimitating the perinotum in the majority of the specimens. The sole is narrow and surpasses the posterior limit of the body when the animal is moving. The width of the sole is always less than the width of the right hyponotum, but never equals less than half its width. The female genital pore is located ventrally, in the posterior half of the right hyponotum, while the male genital pore is located in the anterior region, under the inferior right tentacle.

Internal Morphology (Figures 8–23): Salivary glands formed by large and well differentiated acini. Anterior intestinal loop located behind the digestive gland anterior lobe. One pair of pallial and one of pedal nerves run both parallel and together to each other from the central nervous system toward the posterior portion of the body cavity. They are united (all four) throughout the entire part of the path on the sole and are slightly separated at the posterior region. The pedal aortic artery runs between the pairs of pallial and pedal nerves. It arises from a bifurcation of the anterior aortic artery near the pericardium and runs between the nerves until they terminate at region posterior of the body. The pedal gland is long, flattened, with a conspicuous, median, longitudinal, lighter stripe. The posterior extremity of the gland is free in the body cavity and receives very thin and short ducts in its extremity. The rectum penetrates in the tegument at the height of the female genital pore, behind the oviduct (Figure 12). The anus opens in the posterior region of the body, where the free end of the sole of the foot protects it. An opercular membrane protects the anal opening. The bursa copulatrix is spherical to oval-shaped and presents a short and thickened duet that opens into an atrium, into female genital pore. The canalis junctor penetrates in the bursa itself (not in the bursa copulatrix duct) (Figure 12). At the junction between the bursa copulatrix and the oviduct is a small, yellowish accessory gland completely immersed in the tegument (Figure 12). In some specimens, the accessory gland can be seen by transparency through the tegument. The penial gland presents a short and conical or long papilla with a terminal mammila and 18-26 tubules (Figures 19-22). The tubules located at the base of the papilla in the penial gland are sinuous, not distinguished by size. Some present the extremity or the median region bifurcated. From the posterior region of the penial gland extends the retractor muscle, which is connected to the penis retractor muscle and together these are inserted in the tegument. The penis is robust, with no spathe, with a small base and glans with a wide basal region narrowing toward the apical extremity (asymmetrical arrow shaped penis) (Figures 13–15). The distal extremity of the glans presents a digitiform margin (Figures 16–17). On each side of the basal region of the glans there is a whitish nervure. On the anterior region, the glans presents small projections shaped as minuscule tubercles (Figures 16–18). These are arranged in two, three or more longitudinal rows or are unevenly distributed on the anterior region

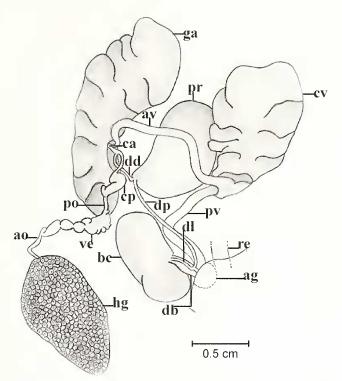
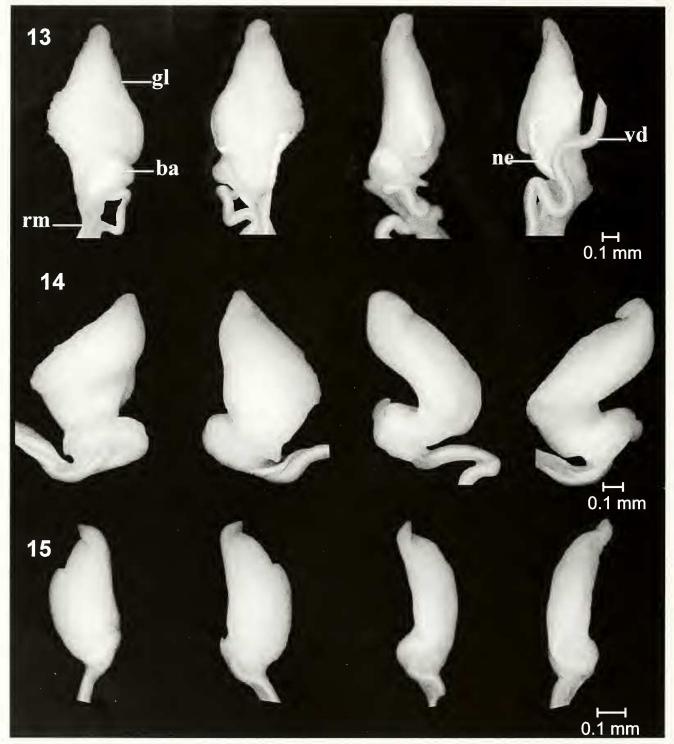


Figure 12. Part of the reproductive system of Belocaulus will-ibaldoi new species (lot 8997). Abbreviations: ag: accessory gland; ao: portion of spermoviduct; av: anterior region of oviduct; bc: bursa copulatrix; ca: "carrefour"; cp: fertilization pouch; cv: coiled region of oviduct; db: bursa copulatrix duct; dd: posterior distal vas deferens; dl: canalis junctor; dp: posterior proximal vas deferens; ga: albumin gland; hg: hermafrodict gland; po: portion of spermoviduct; pr: prostate; pv: posterior region of oviduct; re: part of rectum; ve: seminal vesicle.

of the glans. In young animals, the digitiform margin of the opening may be inconspicuous or not developed. In young specimens the tubercles may also be absent, difficult to see, or even appear as small depressions (future tubercles). The penis and the penial gland are independently surrounded in their own muscular sheath. Both sheaths fuse to form an anterior atrium near the male genital pore (located at the base of the inferior right tentacle) (Figure 23).

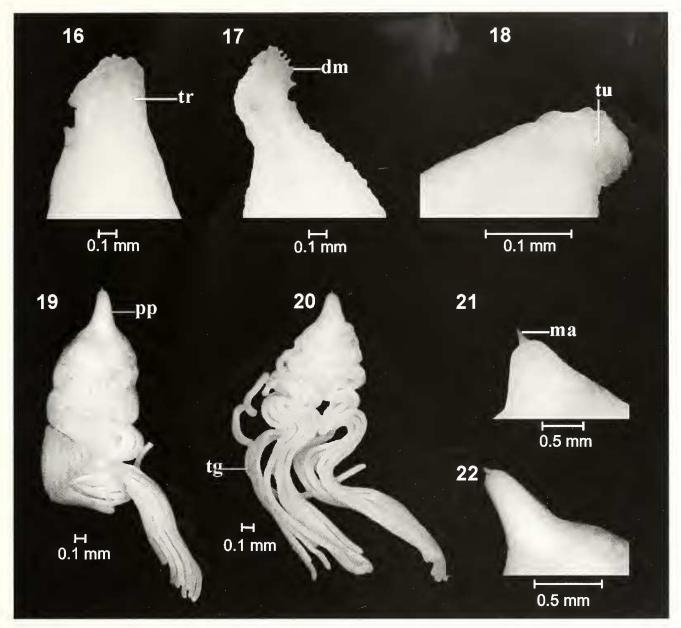
The brown jaw (Figure 8) is located at the dorso-anterior region of the buccal bulb. It forms an arch composed of 21–25 transversal plates, partially covered and parallel to each other, which resemble lathes with a keel-shaped dorsal region; plates are ornamented with strong transversal and weaker longitudinal stripes. The radula (Figures 9–11) is composed of lateral teeth on each side of a central tooth. The radular formula varies from C/1+L52–55/2. The central teeth are small, triangular, and unicuspid. The lateral teeth, larger than the central teeth, are triangular and unicuspid. On the dorsal region of the apical extremity of the lateral teeth the cuspid stands out from the rest of the teeth. The lateral teeth are triangular, but the cuspid is not as prominent as in the teeth closer to the central teeth.



Figures 13–15. Four different views of the penis from three adult specimens of *Beloeaulus willibaldoi* new species. **13.** Lot 8997. **14.** Lot 8995. **15.** Lot 9006. Abbreviations: **ba:** penis base; **gl:** glans; **ne:** whitish nervure; **rm:** penis retractor musele; **vd:** anterior vas deferens.

Measurements (mm): Holotype: 7.1 cm of total length, 1.8 cm of total width, 0.5 cm of width of the sole, 0.9 cm of width of the left hyponotum and 0.9 of width of the right hyponotum. Paratypes (four specimens): to-

tal length from 4.8 cm to 6.3 cm, total width from 1.1 cm to 1.8 cm, width of the sole from 0.3 cm to 0.4 cm, width of the left hyponotum from 0.5 cm to 0.7 cm, and width of the right hyponotum from 0.6 to 0.7 cm.



Figures 16–22. Penis and penial gland of *Belocaulus willibaldoi* new species. 16–17. Distal extremity of the glans (lot 8997). 18. Distal extremity of the glans (lot 8995). 19. Penial gland (lot 8995). 20. Penial gland (lot 8987). 21. Papilla of the penial gland (lot 8997). 22. Papilla of the penial gland (lot 8987). Abbreviations: dm: digitiform margin; ma: mamilla; pp: papilla of penial gland; tg: penial gland tubules; tr: tubercle row; tu: unevenly arranged tubercles.

Type Material: Holotype: MZUSP 87747; four paratypes: MZUSP 87748 (one specimen), MZUSP 87749 (one specimen), MZUSP 87750 (two specimens).

Type Locality: Brazil, São Paulo State, São Paulo, Bairro Parque Fernanda I., 23° 40′ 05.89″ S, 49° 47′ 26.66″ W.

Etymology: The specific name honors Dr. José Willibaldo Thomé for his great contribution to the knowledge of veronicellids and other terrestrial gastropods.

Distribution (Figure 1): Brazil; Minas Gerais State (MG): Rio Acima; São Paulo State (SP): Caieiras, Guarulhos, Osasco, São Paulo; Rio Grande do Sul State (RS): Ernestina, Gravataí, Igrejinha, Porto Alegre, Riozinho, Sapiranga, and Vila Maria; Santa Catarina State (SC): Chapecó.

Habitat and Habit: The specimens of *Belocaulus willibaldoi* collected in MG, SP, SC, and RS were found in urban centers and surrounding areas, in gardens and soil, under tree trunks, wood, plastic and other objects

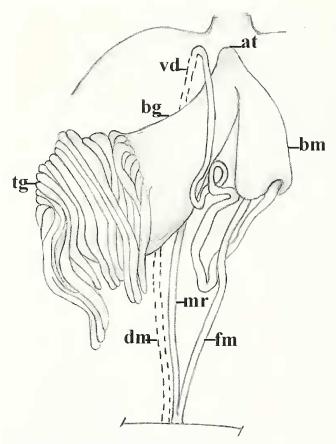


Figure 23. Penial complex of *Belocaulus willibaldoi* new species (lot 8997). Abbreviations: at: common atria; bg: muscular sheath of the penial gland; bm: muscular sheath of the penis; dm: middle vas deferens; fm: penial gland tubules; mr: penial gland retractor muscle; tg: penial gland tubules; vd: anterior vas deferens. Size of complex: 1.8 cm.

on the ground. They are active mainly at night or, after rainy periods, during the day.

DISCUSSION

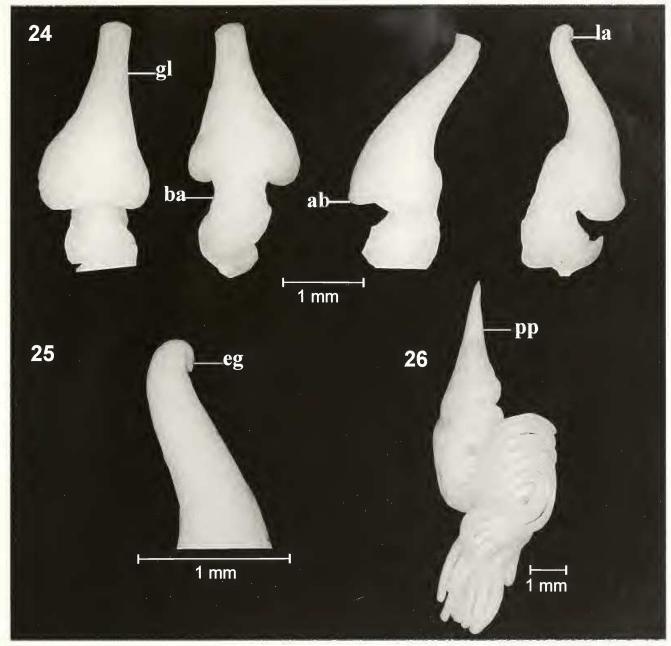
The new species described here from southern and southeastern Brazil is typical of the genus Belocaulus because it presents a penis shaped as an asymmetric arrow and an accessory gland connected to the female genital atrium, which are the two main characters of the genus according to Hoffmann (1925) and Thomé (1975). Sarasinula also includes species with an arrow-shaped penis (Gomes 2007), but penises in Sarasinula tend to be more symmetrical and there is no accessory gland. In addition, Sarasinula species are larger species and they have a more oval shape, with a different pattern of external pigmentation. The new species has relatively small size and is represented by slender slugs when compared to other species found in southern and southeastern Brazil. As *Belocaulus angustipes*, *B. willibaldoi* is a small species when compared to those of other Neotropical genera. It is slender with a strongly narrow sole. Externally, both species cannot be distinguished from each other: their coloration ranges from brown to beige or gray, in different degrees of intensity. In *B. willibaldoi* the width of the sole of the foot is smaller than the width of the right hyponotum, as described by Santos and Thomé (1999) for *B. angustipes*.

Internally, both species are also very similar. There are no differences regarding the digestive, circulatory, and nervous systems. The main differences are observed in the male reproductive system, where the main diagnostic features in Veronicellidae are found (Semper, 1885). Small variations are also observed in the accessory gland, radula, and jaw. The penis of B. willibaldoi is robust, with no spathe, with a small base and a glans, with a wide base narrowing towards the apical extremity with a digitiform margin. The anterior region of the penis bears minuscule tubercles, which can be scattered or arranged in two, three, or more longitudinal rows. The new species is distinguished from Belocaulus angustipes which, according to Pitoni and Thomé (1981) and Santos and Thomé (1999), presents only a short, screw-shaped socket, distal extremity widened and truncated, and glans with rhomboid extremity. In some specimens the glans can be bilobed. In B. angustipes, adjacent to the penis base, the glans is projected backwards over itself (in one side of the penis) (Figures 24-26). In B. willibaldoi the penis base is shorter and less defined than in B. angustipes. In both species a labium is frequently formed on the glans extremity, which folds back covering the opening of the vas deferens.

The penial gland, in general, is similar in both species, differing only in the number of tubules. In *B. willibaldoi*, the penial gland presents from 18 to 26 tubules, while in a *B. angustipes* it presents 13–22 tubules (Pitoni and Thomé 1981; Santos and Thomé, 1999). A terminal mammila was observed in the papilla extremity in *B. willibaldoi*. Even though Pitoni and Thomé (1981) and Santos and Thomé (1999) did not mention the existence of a mammila in *B. angustipes*, it was observed in all specimens examined in this study. This mammila is not, however, so conspicuous due to the fact that the papilla is narrower when compared to that of *B. willibaldoi*.

The accessory gland of *B. willibaldoi* is completely inserted in the tegument, differing from that of *B. angustipes* in which, according to Thomé (1975) and Silva and Thomé (1995), it can be totally or partially covered by the tegument. According to Silva and Thomé (1995), the accessory gland releases a lubricant secretion, probably used during copulation, toward the female genital pore, since it opens in this region.

The morphology of the jaw of *B. willibaldoi* is similar to that described for *B. angustipes* by Thomé and Chaves (1997). The jaw and the radula of both species are distinguished only by the number of jaw plates and the number of teeth per row. According to those authors, the jaw of *B. angustipes* includes 19–22 plates, while *B. willibaldoi* includes 21–25 plates. Based on the characters presented by Thomé and Chaves (1997) for



Figures 24–26. Penis and penial gland of *Belocaulis angustipes* (lot 9030). **24.** Four different views of penis. **25.** Distal extremity of the glans. **26.** Papilla of penial gland; **ab:** glans reflected backward over itself; **ba:** penis base; **eg:** distal extremity of glans, without tubercules; **gl:** glans; **la:** labium; **pp:** papilla of penial gland without a mamilla.

the radula of *B. angustipes*, we notice that the radular formula in *B. willibaldoi* (C/1+L52–55/2) is higher, since *B. angustipes* presents C/1+L34–38/2.

Belocaulus, which was regarded by Pitoni and Thomé (1981) as a monotypic genus, includes another species, B. willibaldoi, which occurrs in the states of Minas Gerais, São Paulo, Santa Catarina, and Rio Grande do Sul. The known records of B. angustipes reach the most southern point within the distribution

of the genus, including localities in Argentina and in Brazil (states of Rio Grande do Sul and Santa Catarina). Although both species have been recorded from Rio Grande do Sul and Santa Catarina, it is probable that *B. angustipes* occurs more to the south, while *B. willibaldoi* more to the north. With the description of *B. willibaldoi*, the distribution range of *Belocaulus* is extended to the states of Minas Gerais and São Paulo, Brazil.

ACKNOWLEDGMENTS

Research performed with financial support from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), grant n° 06/56217-4.

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